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Gamma-glutamyl transferase and cardiovascular disease risk

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ABSTRACT

Cardiovascular diseases (CVD) represent a major health problem in adult population worldwide. Together with conventional risk factors, gamma-glutamyl transferase (GGT) is involved in the pathogenesis of CVD. The purpose of this review is to provide informations about GGT synthesis, physiological roles and the involvement in the atherosclerotic plaque formation. The results of the most recent clinical studies performed to find an association between GGT serum activity and patients with hypertension and coronary artery disease (CAD) are presented.

Keywords: cardiovascular diseases, gamma-glutamyl transferase, atherosclerotic plaque

INTRODUCTION

CVD are the most common cause of death worldwide. In Europe, over 4 million deaths (more than 49%) are caused by coronary heart disease (CHD-20%), stroke (14%) and other CVD (15%) [1].

Major cardiovascular risk factors are represented by:

- age
- dyslipidemia
- diabetes mellitus
- arterial hypertension
- obesity
- smoking
- metabolic syndrome
- excessive alcohol consumption
- systemic inflammation
- oxidative stress (OS)
- genetic factors
- non-alcoholic fatty liver
- comorbidities (chronic kidney disease, coronary calcification)
- and GGT [2].

GGT is a glycoprotein located on the outer surface of the cell membrane. In mammals, this glycoprotein is found as a dimer with a molecular weight of 68kDa which presents two subunits: a large subunit of 46kDa and a small subunit of 22kDa. The large GGT subunit presents an intracellular N-terminal sequence, a hydrophobic transmembrane domain and an extracellular domain, involved in GGT anchoring at the surface of cell membranes. The active center of the enzyme is present at the level of the second subunit, the small one [3].

With the exception of erythrocytes, all cells express GGT [4]. Increased GGT activity has been reported in tissues that exhibit secretory and absorptive function such as kidney, bile system, epididymis and intestine. The maximum activity of the GGT is recorded at the level of the ductal laminar surface of the mentioned tissues [5]. GGT is

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synthesized as a polypeptide chain which undergoes autoproteolytic cleavage with the formation of the two subunits: small and large. Human GGT is encoded by at least seven genes located on chromosome 22, but only one gene leads to functional GGT synthesis. On chromosomes 18, 19 and 20 are found the gene sequences that are nonfunctional or are pseudogenes [6]. Using chromatography techniques, four GGT fractions were identified, which present different molecular weights: big-GGT (b-GGT), medium (m-GGT), small (s-GGT) and the free GGT (f-GGT). b-GGT represents the precursor for the m-GGT and s-GGT fractions, while f-GGT is considered the soluble fraction of the enzyme [7].

PHYSIOLOGICAL ROLES OF GAMMA-GLUTAMYL TRANSFERASE (GGT)

GGT is involved in the transport of amino acids, due to its localization in tissues, *via* the gamma-glutamyl cycle. The most important role of GGT is the degradation of glutathione (GSH), the main intracellular antioxidant with thiol structure in humans [2,8].

GSH is involved in protection against OS, redox signaling, detoxification against xenobiotics, cell proliferation, nitric oxide metabolism, fibrogenesis, apoptosis, sulfur metabolism and sulfur transport and storage. GSH is synthesized in cells' cytoplasm, transported to the extracellular environment where it is degraded by GGT to obtain the dipeptide cysteinyl-glycine and the glutamil residue. The mixed dipeptide is further hydrolyzed into cysteine and glycine [2,8,9].

Cysteine obtained in the extracellular environment is captured by cells and used as an essential precursor for GSH *de novo* synthesis and other proteins. GGT contributes to the maintaining of a optimal concentration of GSH in cells' cytoplasm and in the fight against OS. Increased plasma and urinary concentration of GSH would be due to a GGT deficiency which is extremely rare, being an autosomal recessive disorder or due to alterations in the central nervous system [8,9].

Circulating GGT is produced especially at hepatocyte level, the synthesis being influenced by genetic and environmental factors. GGT is involved in the metabolism of endogenous compounds such as leukotriene C4 and xenobiotics after their conjugation with GSH. These compounds are degraded by GGT with gamma-glutamyl residue formation and conjugated compounds containing the cysteine-glycine dipeptide, which further under the action of peptidases are degraded to mercapturic acids and eliminated *via* the urinary tract [8-10].

GGT plays a crucial role in the body's defense against OS, detoxification and in the inflammatory process [11]. Epidemiologic and clinical studies detected a close association between GGT level and risk of CVD, diabetes mellitus and metabolic syndrome [12-14].

GAMMA-GLUTAMYL TRANSFERASE AND PATHOLOGICAL PROCESSES

Increased serum GGT activity is correlated with a number of systemic disorders such as liver disease and alcohol consumption [2,15,16]. An increased cellular activity of GGT is specific for different types of neoplasms such as liver, lung, prostate or breast cancer. Increased GGT activity, may be due to the exposure to various oxidants. GGT increased level represents the adaptive response of the organism to defend against oxidative and toxic stress [17-20].

GGT increased levels are associated with 30-50% risk of CVD such as:

- hypertensive diseases (Hazard ratios; HR = 1.31)
- ischemic heart diseases (IHD, HR = 1.29)
- total stroke (HR = 1.29)
- acute myocardial infarction (HR = 1.30)
- heart failure (HR = 1.48)
- hemorhagic stroke (HR = 1.42)
- ischemic stroke (HR = 1.27) [21]

GGT AND ATHEROSCLEROTIC PLAQUE

GGT contributes to atheroma plaque formation, being also present in coronary atheroma plaque. GGT mediates extracellular GSH degradation with glutamic acid and Cys-Gly dipeptide formation. This dipeptide under the action of specific dipeptidase undergoes hydrolysis with release of cysteine and glycine residues. O_2 under the action of the dipeptidase accepts a proton and forms a reactive species of oxygen (ROS), the superoxide anion O_2O_2 in contact with a proton generates hydrogen peroxide (H_2O_2), which oxidizes cholesterol from LDL. Ox-LDL will accumulate in the arterial wall, foam cells development and atheroma plaques formation. Hemodynamic changes, OS, inflammation, together with conventional risk factors for coronary artery disease (smoking, diabetes, obesity) lead to atherosclerotic plaque rupture and thrombosis [22].

$$\text{GSH (intracellular space)} \xrightarrow{\text{translocation}} \text{GSH (extracellular space)}$$

$$\text{GSH (extracellular space)} \xrightarrow{\text{GGT}} \text{Glu} + \text{Cys} - \text{Gly}$$

$$\text{Cys} - \text{Gly} \xrightarrow{\text{dipeptidase}} \text{Cys} + \text{Gly}$$

$$O_2 \xrightarrow{\text{Cys}-\text{Gly}} O_2^{\cdot} + {}^{\cdot}\text{CYS}-\text{GLY}$$

$$H^+ + O_2^{\cdot} \rightarrow H_2O_2$$

$$\text{LDL} \xrightarrow{\text{peroxidation reactions}} \text{ox} - \text{LDL}$$

$$\text{cardiometabolic risk factors} + \text{ox} - \text{LDL} \rightarrow \text{endothelial disfunction}$$

$$monocytes \xrightarrow{CVD\ risk\ fcators} colagen, elastin\ degradation \xrightarrow{+ox-LDL} foam\ cells$$

$$foam\ \text{cells} \rightarrow \text{atherosclerotic plaque formation} \rightarrow \text{plaque instability} \rightarrow \text{mortability}$$

FIGURE 1. *GGT and atherosclerotic plaque (adapted from [2,22])*

Jeon J et al. conducted an observational study investigating the relationship between GGT and atherosclerotic CVD (ASCVD) in 419,433 Korean adults. GGT increased serum activity was detected at ASCVD and hemorrhagic stroke patients [23]. Immunohistochemical and histochemical studies have observed enzymatic activity of GGT in coronary atheroma plaque [24]. In contrast, Saely Ch et al. detected an association between liver enzymes (alaninaminotransferase ALT, ALT / AST- aspartateaminotransferase ratio and GGT) and metabolic syndrome. These enzymes were not associated with coronary atherosclerosis determined by angiography in 1.000 patients who were suspected or diagnosed with CAD [25]. Shimizu Y et al. conducted an observational study of 562 Japanese people aged 60-69. GGT correlated positively with atherosclerosis patients who presented an higher number of CD-34 positive cells. In patients with low CD34-positive cells, GGT correlated with hypertension [26].

GGT AND HYPERTENSION

Hypertension is one of the most common risk factors for CVD worldwide. In a three-year study conducted by Cheung et al. which included 235 hypertensive individuals and 708 healthy volunteers, plasma levels of GGT, ALT and alkaline phosphatase (ALP) were detected. The results of the study confirm that only GGT is an independent predictor of hypertension [27].

The study conducted by Jung CH et al. that included 10.988 participants observed a positive correlation between GGT, systolic and diastolic blood pressure, body mass index, waist circumference, total cholesterol, fasting plasma glucose, LDL, triglycerides, uric acid and high-sensitivity C-reactive protein (CRP) levels. Increased GGT levels above the normal limit is a major risk factor for hypertension, especially in the case of drinkers and alcoholics, but also in the normoweight individuals [28]. Saijo Y et al. suggested a possible connection between GGT and arterial stiffness [29].

Buzdugan et al. conducted a study that included 409 patients divided into 3 groups: group 1 with hypertensive patients, the second group included prehypertensive patients and the control group. GGT activity, neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio were determined. GGT values were independently associated with both hypertensive and prehypertensive patients [30].

Bozkus F et al. detected increased levels of GGT in hypertensive patients with moderate and severe forms of obstructive sleep apnea syndrome. The results of the study indicate a correlation between high serum GGT levels and hypertension in patients with obstructive sleep apnea [31].

Hypertension is a risk factor for metabolic syndrome, so Franzini M et al. investigated the correlation between GGT fractions in hypertensive patients. The study included 90 patients, where GGT fractions were evaluated using chromatographic techniques. Metabolic syndrome was detected in 36% of the patients included in the study. Fractions b and f of GGT were positively correlated with body mass index, glucose level, triglycerides and insulin. A negative correlation was observed in relation to HDL. b-GGT fraction values increase at the same time with the increasing degree of liver steatosis [32].

GGT AND CORONARY ARTERY DISEASE

CAD is characterized by atherosclerosis in coronary arteries, a major cause of death and disability in developed countries. Hank K et al. observed a positive association between patients with coronary artery stenosis and lipid parameters (total cholesterol, triglycerides, LDL, HDL), CRP, homocysteine, GGT and fibrinogen. With the exception of HDL, all of the mentioned parameters showed increased values in patients with arterial stenosis versus the control group. The mentioned parameters may be independent risk factors of coronary artery stenosis in elderly patients with coronary heart disease (CHD) [33].

Ndrepepa G et al. investigated the correlation between GGT activity and CAD at 5501 patients undergoing percutaneous coronary intervention. GGT activity was increased in this category of patients, being a risk factor for prediction of all-cause and non-cardiac mortality, but not cardiac mortality [34].

Sheikh M et al. detected the serum GGT level in 367 patients with premature CAD. The cross-sectional study carried out reported an increased statistical level of GGT in CAD patients versus the control group. GGT may be used as a predictive parameter for premature arterial disease in young patients [35].

Zen YY et al. conducted a cohort study of 5638 patients over an 8-year period (2008- 2016) to demonstrate whether the GGT to albumin ratio can be an independent marker of mortality and bleeding events in CAD during percutaneous coronary intervention. The low albumin ratio is associated with a high number of mortality and bleeding events compared with patients who presented an increased albumin ratio. In patients with acute coronary syndrome presenting a high albumin ratio, the risk of bleeding events decreased by 57.3%.

The study observed in patients with stable CAD a decrease in the risk of mortality from different causes by 28.6% in patients with high albumin ratio. The albumin ratio was considered as an independent and new predictor of mortality and bleeding events in patients with CAD undergoing percutaneous coronary intervention [36].

The same team of researchers presented in 2019 the results of a study performed in patients with CAD and cardiac failure, regarding the serum GGT level after percutaneous coronary intervention. 5,638 patients were divided into 3 groups according to GGT tertiles: first group included 1,875 patients and GGT < 19.6 U/l; the second group consisting of 1,880 patients and GGT values between 19.6 and 32.9 U/l; the last group included 1,883 patients and a GGT value ≥ 32.9 U/l. The incidence of HF in the first group was 3.3%, group 2 of 2% and in the last group of 3.5%. Serum GGT levels were independently associated with HF after percutaneous coronary intervention. A serum GGT level lower than 19.6 or greater than 32.9 U/l increases the risk of HF in patients with CAD who undergo percutaneous coronary intervention [37].

Arasteh S et al. conducted a study that included 500 patients with CAD to investigate a possible association between serum GGT and stenosis severity. The results of the study showed a positive association between serum GGT activity and CAD patients. GGT level was increased in patients with more than 50 obstructions compared to the healthy group or in patients with less than 50% coronary artery obstruction. The increased level of GGT is considered a predictive biomarker of stenosis severity in CAD patients [38]. Bharani V et al. investigated the relationship between GGT and 200 CAD patients after coronary angiography. Increased GGT levels were positively correlated with total cholesterol, triglycerides and VLDL.

The results of the study showed that the most important risk factors for CAD are:

- dyslipidemia 93%
- metabolic syndrome 59.5%
- hypertension 54%
- obesity 47.5%
- diabetes 46 %
- tobacco consumption 19.5%
- smoking 17%
- family history of CAD 10% [39]

Ndrepepa G et al. investigated the association between GGT and arterial fibrillation in 5,501 CAD patients. Patients with arterial fibrillation presented elevated serum GGT levels compared to patients with sinus rhythm; 52 U/l versus 34.8 U/l. The increased activity of GGT is independently associated with arterial fibrillation in CAD patients [40].

GGT was also detected in patients diagnosed with diabetes mellitus and CAD. The aim of the study conducted by Ndrepepa G et al. was to observe an association between GGT activity and the mortality rate of diabetic patients and CAD who underwent percutaneous coronary intervention.

Patients were divided into 3 groups according to the GGT value:

- group 1: GGT <29.4 U/l, n = 484 patients
- group 2: GGT> 29.4-52.5 U/l, n = 479 patients
- group 3: GGT> 52.5 U/l, n = 482 patients

The results of the study recorded 179 deaths as follows: 46 (11.9% – group 1), 49 (12.1%– group 2) and 84 (21.4% – group 3). Cardiac death was reported in 101 patients: 22 (5.8% -group 1), 30 (7.2% -group 2) and 49 (12.9% -group 3). GGT activity greater than 52.5 U/l is associated with increased mortality and cardiac death in patients with diabetus mellitus and CAD [41].

CONCLUSIONS

GGT is a glycoprotein expressed by almost all human body cells, implicated in GSH metabolism, detoxification and inflammatory processes. Increased GGT activity is correlated with a number of systemic diseases in adult population such as CVD, liver disease, diabetes, and cancer. GGT contributes to atheroma plaque formation by ROS generation that will cause LDL oxidation. Serum GGT level is increased in hypertensive and CAD patients. GGT can be considered a valuable biomarker for predicting and monitoring different CVD.

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